



Genetic diversity in the Ethiopian garden cress (*Lepidium sativum* L.) using microsatellite markers

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ABSTRACT

Diversity has not been exhaustively studied in the Ethiopian garden cress (*Lepidium sativum* L.). Therefore, the objective of the present study was to determine the genetic diversity among garden cress genotypes using microsatellite or simple sequence repeat (SSR) markers. One hundred twelve garden cress genotypes collected from diverse growing regions of Ethiopia were investigated using 12 SSR markers which were earlier developed for closely related *Lepidium subulatum*. A total of 1387 alleles were identified, with the average of 116 alleles per SSR marker. The average polymorphism information content (PIC), Shannon diversity index and Nei's expected heterozygosity were 0.444, 0.750 and 0.443, respectively. High levels of Shannon diversity were noted within population (0.696) than between populations (0.304). Analysis of Molecular Variance (AMOVA) also confirmed that 79% and 21% of total variations were attributed to the within- and between-populations, respectively, indicating greater exchange of gene pool across regions of origin. The genetic distance between populations ranged from 0.044 to 0.396. Cluster analysis using un-weighted neighbor joining method revealed five clusters. The Principal Coordinate Analysis (PCoA) showed the distribution of genotypes in the scatter-plot was highly dispersed at 22% of the total variation, demonstrating complex genetic relationship among genotypes of different geographic origin. Genetic distance matrix among nine populations revealed three different groups to be used as divergent populations in the future breeding programs. Hence, these markers were effective in studying genetic diversity in the Ethiopian garden cress genotypes. Although the transferability of SSR markers from related species was found to be high, the efficiency of identifying more polymorphisms will be improved using garden cress specific markers.

1. Introduction

Landraces have been shown to be excellent sources of genes for novel alleles [1]. Garden cress (*Lepidium sativum* L.) which belongs to the Brassicaceae Family is unexploited for medicinal purposes, oil and is an important vegetable crop. Variability in garden cress is largely based on its morphology, agronomic properties, and biochemical traits characterized by low level of polymorphism and highly influenced by their environment [2,3].

Characterization and evaluation of germplasm variability using molecular markers is becoming important to develop strategies for conservation and collection, and to increase the sustainable utilization of plant genetic resources [4]. Molecular genetic diversity assessment may not correlate with phenotypic expression of a genomic trait.

Nevertheless, it offers numerous advantages over the conventional, phenotype-based alternatives as the former is stable and detectable in all tissues regardless of growth, differentiation, development, or defense status of the cell as it is not affected by environmental, pleiotropic and epistatic effects [3,5]. Molecular markers are more efficient, precise and reliable in discriminating closely related species and cultivars [2,4–6]. Inter simple sequence repeat (ISSR) of garden cress have been previously studied by Kuar et al. [7] and Said and Kassahun [8]. However, limited numbers of polymorphic markers were used and more importantly the ISSR marker is not as robust, highly polymorphic, co-dominant markers as Simple Sequence Repeat (SSR) marker. As a result, the assessed number of genotypes and the level of efficiency, amount of information generated, and number of polymorphic markers seem insufficient for detailed genetic diversity studies.

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Simple Sequence Repeat (SSR), also commonly known as microsatellite, marker is particularly more valuable than other molecular markers such as Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) as the former is more variable, transferable, co-dominant, robust, chromosome specific and multi-allelic in nature than the latter [4,9,10]. Earlier studies using SSR markers confirmed their significance in assessing the genetic variability and distinguishing among closely related genotypes of Brassica germplasm [11,12]. Hence, SSR marker in combination with agro-morphological markers has great discriminatory power to differentiate divergent populations [13]. So far, to our knowledge, no study was made on molecular diversity of garden cress using SSR marker. Therefore, the present study was undertaken to evaluate the genetic diversity and the relationship of 112 garden cress genotypes using microsatellite markers.

2. Materials and methods

2.1. Plant materials and genomic DNA isolation

Detailed information on the origin of plant materials used in this study are presented in Table 1. These include the name of State, Zone and District or Woreda where collections were made as well as geographical coordinates and altitude, for those available. One hundred and twelve garden cress genotypes collected from diverse growing regions in five regional states were used for the study. The molecular analysis was conducted in 2017 at Molecular and Biotechnology Laboratory of the National Agricultural Biotechnology Research Center (NABRC), Holetta, Ethiopia. Genomic DNA was isolated by grinding 0.5 g of dry seeds into fine powder using pre-chilled mortar and pestle and transferring the powder into the 2 mL eppendorf tube with 1.3 mL DNA extraction buffer (1 M NaCl, 50 mM Tris-HCl (pH 8.0), 50 mM EDTA (pH 8.0), 1% PVP) as described by Liu et al. [14] with some modifications. The quantity and quality of DNA were tested by running the sample containing 5 µL gDNA, 2 µL of 6X loading dye in 1xTAE buffer electrophoresis for 30 min at 100 V. A 0.8% agarose gel stained with 2 µL gelRed was used. The pictures of the gel were taken on gel documentation system (3UV bench top, M-20 transilluminator). The quantity and purity of genomic DNA were confirmed by a NanoDrop spectrophotometer (ND-8000, Thermo Scientific). DNA samples were then diluted, and normalized at a concentration of 40 ng/µL for use in PCR amplification.

2.2. Cross transferability of microsatellite markers and their optimization

The 12 microsatellite markers earlier designed for *Lepidium subulatum* [13] were also proved to show high level of polymorphism and transferability to the Ethiopian garden cress (*Lepidium sativum*) (Table 2). Their length ranged from 20 to 22 bp. The observed allele sizes of PCR products of the current study ranged from 50 to 1000 bp as earlier reported for other species [15,16]. Detailed information related to the 12 SSR markers is shown in Table 2. Touchdown (TD) PCR programs were used as described by Hecker and Roux [17] for optimizing these SSR markers by adjusting the concentrations of the primers and template DNA. Ten randomly selected genotypes were used for the optimization of PCR conditions and for testing the reproducibility of PCR product patterns before analyzing all genotypes.

2.3. PCR amplification

The PCR reactions were performed in a final volume of 10 µL which contains 2 µL genomic DNA (from 40 ng/µL stock), 1.5 µL forward and reverse primer (from 10 µM stock), 5 µL of GoTaq2 green master mixture and 1.5 µL nuclease free water. PCR amplifications were performed in a peqSTAR Thermal Cycler (United Kingdom) using different

programs mainly based on the melting temperatures of the set of primers (Table 2). The standard PCR amplification follows the following condition: initial denaturation at 94 °C for 4 min; followed by 35 cycles of a denaturation step at 94 °C for 1 min, an annealing step for 1 min, and first extension step at 72 °C for 2 min; and the last extension at 72 °C for 7 min. Specific conditions set for the standard and touchdown (TD) PCR programs are shown in Table 2.

2.4. Agarose gel electrophoresis

Five µL of PCR amplified products were loaded on 3% electrophoretic grade agarose gel with gelRed which visualizes DNA fragments. The gel was run at 100 V for 2 h and 30 min in 1X TBE buffer using standard horizontal electrophoresis. A 25/100 bp DNA ladder (BIONEER) were used as a DNA molecular size standard. The ladder contains 25–2000 discrete fragments. Fragments were detected and photographed using a UVP gel documentation system (Ultra-Violet Products Ltd., Cambridge, United Kingdom) as described above (Fig. 1). All SSR fragments were scored manually and converted into binary data, i.e., 1 for the presence of the product and 0 for the absence of the product. Consistent and reproducible PCR products were scored as present (1) and absent (0). Smeared and weak bands were excluded. Fragments of the same molecular weight were considered to represent the same locus.

2.5. Data analysis

The performance of the SSR markers was measured using Polymorphic Information Content (PIC) which was calculated using the formulas: $PIC = 1 - \sum P_i^2$, where P_i is the frequency of the population carrying the i th allele, counted for each SSR locus [18]. To study population structure, samples were pooled into nine geographic groups (populations) based on the location of collection and magnitude of common boundary they share to form seemingly homogenous groups except in Pop6 for which information on sites of accession collections are not described (Table 3). Analyses were observed at two levels (1) within populations (combined populations); and (2) among populations. The data were analyzed using POPGENE version 1.32 [19] to calculate the genetic parameters such as the percent (%) polymorphism, genetic diversity or heterozygosity ($H = \text{Nei's gene diversity}$); Shannon's index (I) and gene flow estimation (N_m) ($N_m = 0.25 \times (1 - F_{ST}) / F_{ST}$) [20]. The Shannon diversity index (I) calculated using the formula: $H_o = -\sum \pi_i \ln \pi_i$; where π_i is the frequency of a given SSR fragment. H_o was calculated at two levels: the average diversity within populations (H_s) and the total diversity (H_t) [21].

Diversity among populations was estimated as $(H_t - H_s) / H_t$ [22]. The proportion of diversity between populations ($H_t - H_s$), relative to total diversity (H_t) was measured to represent the relative degree of genetic differentiation between populations. H_s is the mean diversity of each of the populations, and H_t is the total diversity of the populations [23]. Pairwise comparisons of the populations and genotypes were used to generate genetic similarity coefficient. Genetic similarity was obtained by Dice algorithm which was described by Nei and Li [24]: Similarity (F) = $2N_{ab} / (N_a + N_b)$; where N_a and N_b are the number of scored fragments for respective individual, N_{ab} is the number of common fragments between individuals 'a' and 'b'. The similarity matrices converted to dissimilarity matrix were subjected to cluster and principal coordinates analysis based on un-weighted neighbour joining method with DARwin version 6 software [25]. To ascertain the statistical strength of genetic relationships identified through this analysis, bootstrapping of the data by 500 was performed. The genetic distance between genotypes/populations was estimated using unbiased Nei coefficient [26]. The genetic variability at different hierarchical levels (among and within the populations) was tested using Analysis of Molecular Variance (AMOVA) implemented in Genalex Version 6.5 [27].

Table 1

The origin of Ethiopian garden cress genotypes investigated in this study.

Germplasm		Area of collection					
Code	Accession No.	State	Zone	Woreda	Latitude	Longitude	Altitude
62	229799	Amhara	East Gojam	Enbise Sar Midir	14°14'00"N	38°39'00"E	2550
75	229798	Amhara	East Gojam	Hulet Ej Enese			2250
104	CG14	Amhara	North Gojam	Goncha Siso Enese			
21	235892	Amhara	North Gondar	Addi Arkay	13°38'00"N	37°14'00"E	2270
33	214243	Amhara	North Gondar	Debark			
42	205163	Amhara	North Gondar	Debark			2820
88	205162	Amhara	North Gondar	Debark			
51	208030	Amhara	North Gondar	Gondar Zuria	12°40'00"N	37°23'00"E	
102	CG12	Amhara	North Shewa	Efratana Gidim			
19	229203	Amhara	North Shewa	Lay Betna Tach Bet			2620
28	229202	Amhara	North Shewa	Lay Betna Tach Bet			2600
38	229200	Amhara	North Shewa	Lay Betna Tach Bet			2150
73	229201	Amhara	North Shewa	Lay Betna Tach Bet	07°12'36"N	40°33'45"E	2310
77	229204	Amhara	North Shewa	Lay Betna Tach Bet	09°04'00"N	36°36'00"E	2580
60	229199	Amhara	North Shewa	Siyadebrina Wayu Ens			2220
26	229205	Amhara	North Shewa	Weremo Wajetuna Mid			2690
10	241777	Amhara	North Wello	Guba Lafto	11°48'00"N	39°37'00"E	2110
1	207542	Amhara	South Gondar	Kemekem			
86	90004	Amhara	South Gondar	Tach Gayint	11°36'00"N	38°34'00"E	2400
61	90018	Amhara	South Wello	Debresina	08°52'00"N	35°04'00"E	2520
97	CG7	Amhara	South Wello	Debresina			
64	90020	Amhara	South Wello	Dessie Zuria	07°09'67"N	35°49'85"E	2000
63	212628	Amhara	South Wello	Kutaber			2400
43	215714	Amhara	South Wello	Werebabu	11°20'00"N	39°47'00"E	2200
85	215713	Amhara	South Wello	Werebabu	11°20'00"N	39°47'00"E	2580
101	CG11	Amhara	South Wello	Werebabu			
112	CG22	Amhara	South Wello	Werebabu			
31	216885	Oromia	Arssi	Merti			1570
47	216886	Oromia	Arssi	Merti			1570
105	CG15	Oromia	Arssi	Tiyo			
110	CG20	Oromia	Bale	Meliyu			
20	237991	Oromia	Bale	Adaba			2430
94	CG4	Oromia	Bale	Agarfa			
95	CG5	Oromia	Bale	Dinsho			
99	CG9	Oromia	Bale	Gaserana Gololcha			
48	19001	Oromia	Bale	Ginir	07°12'36"N	40°33'45"E	2128
14	212852	Oromia	Bale	Goro			
68	19002	Oromia	Bale	Goro	11°20'00"N	39°47'00"E	2164
72	212853	Oromia	Bale	Goro			
41	90002	Oromia	Bale	Sinanana Dinsho			
96	CG6	Oromia	Bale	Sinanana Dinsho			
87	230524	Oromia	East Hararghe	Girawa			
100	CG10	Oromia	East Shewa	Akaki			
11	90006	Oromia	East Hararghe	Deder			
71	90005	Oromia	East Hararghe	Deder	08°34'00"N	34°51'00"E	
15	230831	Oromia	East Hararghe	Girawa	09°01'00"N	41°58'00"E	2050
3	208693	Oromia	East Hararghe	Gursum	09°18'00"N	42°23'00"E	
34	216816	Oromia	East Hararghe	Gursum			
78	230830	Oromia	East Hararghe	Jarso	06°04'00"N	37°17'00"E	2450
79	208669	Oromia	East Hararghe	Kersa	09°10'00"N	41°32'00"E	2180
56	234828	Oromia	East Wellega	Diga Keka			
98	CG8	Oromia	Jimma	Limu Seka			
90	18843	Oromia	North Shewa	Debre Libanos			2241
57	208666	Oromia	West Hararghe	Mieso	14°08'00"N	38°47'00"E	
81	18841	Oromia	West Shewa	Bako	09°12'17"N	37°12'16"E	2440
52	90021	Oromia	West Shewa	Cheliya	09°04'00"N	36°36'00"E	2620
49	19000	Oromia	West Hararghe	Chiro	09°04'38"N	40°56'55"E	1912
92	CG2	Oromia	West Hararghe	Chiro			
93	CG3	Oromia	West Hararghe	Gemechis			
84	208667	Oromia	West Hararghe	Habro	08°56'00"N	40°36'00"E	1900
109	CG19	Oromia	West Hararghe	Mesela			
91	CG1	Oromia	West Shewa	Wolmera			
36	90022	Oromia	West Wellega	Dale Lalo	08°52'00"N	35°04'00"E	1480
2	208769	Oromia	West Wellega	Sayo			1900
7	215808	Oromia	West Wellega	Sayo	08°32'00"N	34°46'00"E	1950
46	215807	Oromia	West Wellega	Sayo	08°34'00"N	34°51'00"E	1720
5	205141	SNNP	Gurage	Goro			1900
39	242916	SNNP	Keficho Shekicho	Chena	07°09'67"N	35°49'85"E	2200
18	240396	SNNP	Keficho Shekicho	Decha	07°13'64"N	36°15'59"E	1950
74	240397	SNNP	Keficho Shekicho	Decha	09°04'38"N	40°56'55"E	
6	202116	SNNP	Keficho-Shekicho	Ginbo			
107	CG17	SNNP	North Omo	Basketo			

(continued on next page)

Table 1 (continued)

Germplasm		Area of collection					
Code	Accession No.	State	Zone	Woreda	Latitude	Longitude	Altitude
12	225725	SNNP	North Omo	Bonke			
103	CG13	SNNP	North Omo	Damot Weyde			
4	8604	SNNP	North Omo	Damot Weyde			2050
58	240808	SNNP	North Omo	Damot Weyde			1950
30	90016	SNNP	North Omo	Gofa Kuria			
106	CG16	SNNP	North Omo	Goffa zuria			
53	225799	SNNP	North Omo	Kemba	06°04'00"N	37°17'00"E	2650
108	CG18	SNNP	North Omo	Mareka Gena			
55	240578	SNNP	North Omo	Melokoza			
35	230829	Somali	Jigjiga	Jigjiga			1980
54	230523	Somali	Jigjiga	JigKigjiga			
70	216815	Somali	Jigjiga	JigKigjiga	14°14'00"N	38°09'00"E	
76	231210	Somali	Jigjiga	Jigjiga	12°40'00"N	37°23'00"E	1910
23	233982	Tigray	Central Tigray	Adwa	14°12'00"N	39°21'00"E	2210
44	237512	Tigray	Central Tigray	Adwa	14°11'00"N	38°52'00"E	1900
50	219961	Tigray	Central Tigray	Adwa			
69	207910	Tigray	Central Tigray	Adwa	14°11'00"N	38°52'00"E	
32	219958	Tigray	Central Tigray	Laelay Maychew	14°08'00"N	38°47'00"E	2130
27	233984	Tigray	Central Tigray	Werielehe	14°05'00"N	38°58'00"E	1860
25	233983	Tigray	Central Tigray	Werie Lehe	14°07'00"N	38°57'00"E	1950
9	238273	Tigray	Central Tigray	Adwa	14°02'00"N	38°04'00"E	2150
13	219962	Tigray	Central Tigray	Naeder Adet	14°03'00"N	38°41'00"E	1700
89	233981	Tigray	East Tigray	Ganta Afeshum	14°12'00"N	39°18'00"E	2140
59	242609	Tigray	East Tigray	Ganta Afeshum			
24	234355	Tigray	East Tigray	Ganta Afeshum	14°13'00"N	39°15'00"E	2090
37	233986	Tigray	East Tigray	Wukro	14°14'00"N	38°39'00"E	2080
111	CG21	Tigray	West Tigray	Shire			
45	233985	Tigray	West Tigray	Laelay Adiyabo	14°14'00"N	38°09'00"E	1910
17	219959	Tigray	West Tigray	Medebay Zana			
8	219960	Tigray	West Tigray	Tahtay Koraro	14°04'00"N	38°15'00"E	1940
16	90012	Unknown	Unknown	Unknown			
22	233679	Unknown	Unknown	Unknown			
29	233370	Unknown	Unknown	Unknown			
40	240579	Unknown	Unknown	Unknown			
65	90010	Unknown	Unknown	Unknown			
66	90009	Unknown	Unknown	Unknown			
67	90014	Unknown	Unknown	Unknown			
80	90017	Unknown	Unknown	Unknown			
82	90007	Unknown	Unknown	Unknown	10°40'00"N	38°56'00"E	
83	90008	Unknown	Unknown	Unknown			

SNNP = Southern Nation and Nationality of People Regional State; Unknown: accessions collected from Ethiopia but exact location was not described.

Table 2

SSR markers used in the current study and PCR conditions.

Marker	Primer sequence (5'→3')	Tm (°C)	Motif Repeat	Expected Product size (bp)	PCR condition
Lsub01	F: CTTTCTCTGCTGAGCTGTCAA R: TTGTCTCTGCCGAAATCCAT	56.4 58.4	(GA) 12	201	*TD 60-50 (10x and 25x)
Lsub02	F: GGATTTAATTCGTGGACAGCA R: CACCGACTACTCCGATCCTC	57.5 62.5	(AG)9	209	**Standard PCR at 55 °C
Lsub03	F:CAAATGAAAGCAGATCAAGCA R: TGGATCAATTTCCTGTTGGA	54.3 55.4	(AG)12	182	Standard PCR at 55 °C
Lsub04	F: TCCATTGATATCCGAGCAA R: GGGTTACGTGATTAGGGAACA	54.3 60.1	(TCA)22	202	TD 60-55/ 53.5 (10x and 25x)
Lsub05	F:GGGTTTGTCCCAACAAGAAGA R: CAGGTCAATCGCGTGTCTTA	56.4 58.4	(GA)9	293	TD 58-49 (9x and 26x)
Lsub07	F:CCAATCAATACCATCTCCCAAG R: TGTCGTTAGAATCTTGCTGAATGT	60.1 60.3	(TG)10	174	TD 60-50 (10x and 25x)
Lsub08	F: GCCAACGTACAACGGAGAAT R: ATCCGATTTCTGCTACTCTGC	58.4 58.4	(GA)10	184	TD 66-57 (9x and 26x)
Lsub09	F: AATGGTGGGCTCGGATTTA R: CCTTTGTTGATTCCTCAATG	55 56.4	(TC)8	171	TD 60-50 (10x and 25x)
Lsub10	F: TGGTGGAGAGGACAAAGGAT R: TCAACGTAAAGCAACCCAAA	54.3 58.4	(GA)8	273	TD 60-52 (8x and 27x)
Lsub11	F:ACTCCGATAAATTTGGGCATC R: CAAATCTCCATTCTCGACCA	56.4 57.5	(AG)8	182	TD 58-49 (9x and 26x)
Lsub12	F: AGCTGGAGATCCGAAGAACA R: TCCATTGAAACCTCAACGTG	56.4 58.4	(GAA)9	181	Standard PCR at 55 °C
Lsub13	F: GCCGAATAAGAGGAGTTGC R: CGCCACTCTAAGCTCTCAC	60.5 62.5	(AG)8	152	TD 66-57 (9x and 26x)

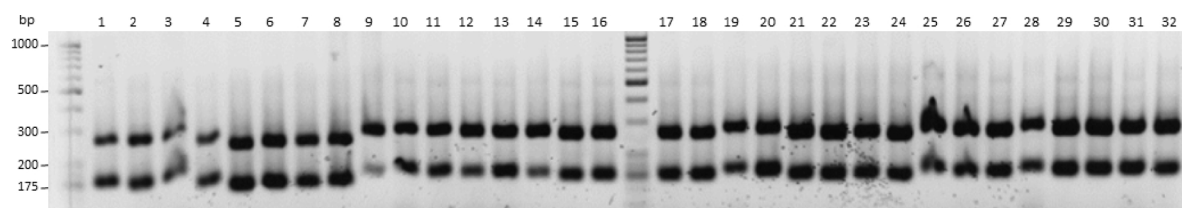


Fig. 1. A gel picture showing the PCR products from 32 randomly selected genotypes using Lsub02 marker. Each lane was loaded with 5 μ l of PCR products. Detail descriptions for the 32 genotypes which correspond to codes are indicated in Table 1.

Table 3

The assembled populations and geographic regions of collection used in SSR marker analysis.

Population	Number of tested	Geographical region of collection
Pop1	14	Arsi and Bale
Pop2	17	Central, East and West Tigray
Pop3	18	Jigjiga and East and West Hararghe
Pop4	8	East Gojam and Wellega
Pop5	14	East, North and West Shewa
Pop6	10	Unknown
Pop7	15	Jimma, North Omo, and Kefficho-Shekicho
Pop8	7	North and South Gondar
Pop9	9	North and South Wello

Unknown: accession collected from Ethiopia but exact location was not described.

3. Results and discussion

3.1. SSR polymorphism and genetic diversity

The observed allele sizes of PCR products of the current study ranged from 50 to 1000 bp. They were not in accordance to earlier report for *Lepidium subulatum* which ranged from 152 to 293 bp. This wide range in the amplicon size was registered for only Lsub10 and Lsub11 markers. Although we repeated the experiments for these two markers, similar range in the amplicon size were obtained. The deviations from the expected amplicon size might be due to, (i) the PCR primers might not be specific for the species under study since they were originally designed for different species in the same Genera, and (ii) genetic variations among garden cress genotypes might be large enough to reveal huge variability in the range of amplicon size. It might also be due to significant influence by the number of mismatches between the two species, the coexistence of the annealing site of SSR primers, the GC-content within the amplified region, degree of encoded protein conservation, length of the primers, and the genetic proximity or the relatedness of the target species [15,16]. The details of SSR loci analyzed and the data on polymorphisms, including number of alleles detected per locus (Na), effective number of alleles per locus (Ne), major allele frequency, Polymorphism Information Content (PIC), observed heterozygosity (Ho), expected heterozygosity (He), gene flow among genotypes (NM), are presented in Table 4. In the present study, the 12 SSR loci produced a total of 1387 alleles with an average of 116 alleles per marker. Number of alleles detected per locus across genotypes ranged from 65 (for Lsub13 marker) to 181 (for Lsub 05 marker). Relatively higher number of alleles per SSR marker is presumably due to the tetraploid nature of the garden cress genotypes under investigation as earlier reported for *Poa pratensis* [28]. The numbers of alleles per loci varied from 2 to 5 with an average of 2.83 alleles per locus were used to estimate the genetic diversity among the 112 garden cress genotypes. The average number of fragments per primer-set were estimated to be 9.63 alleles. These ranged from 6.6 (for Lsub11 marker) to 11.9 (for Lsub10). The high number of alleles per genotype might be due to the heterogeneity in garden cress genotypes used in the current study. These genotypes are admixture of diploid and tetraploid (though not determined by flow cytometry) as well as predominantly cross-

pollinated crops. Such nature of the study materials and the very nature of codominant marker (SSR markers) expected to have higher number of alleles.

Similarly, PIC which represents the allelic diversity for a specific locus, ranged from 0.134 to 0.733 with the mean value of 0.444. Although the mean effective allelic number (Ne) was 2, the values for 12 SSR markers ranged from 1.155 to 3.628. While the mean observed heterozygosity (Ho) was 0.243 that of expected heterozygosity (He) was almost twice of this value (0.446) (Table 4). The low Ho value compared to He might be due to inbreeding nature of the garden cress genotypes under unfavorable environmental condition. On the contrary, Nei expected heterozygosity [29] with the average of 0.443 indicated substantial gene diversity among genotypes. The expected heterozygosity accounts for the frequency of the different types of alleles or loci in the population [30]. In most cultivated plant species, mean heterozygosity values reported from earlier studies were 0.300 for soybean [31], 0.4 for mung bean [32], 0.5 for rice [33] where the latter two were implemented using SSR markers.

The PIC value which represents the relative informativeness of each marker ranged from 0.134 for marker Lsub04 to 0.733 for marker Lsub10. The PIC describes diversity within intra-population diversity and characterizes the degree of polymorphism in each locus, a PIC value of less than 0.25 indicating low polymorphism, and a value between 0.25 and 0.5 shows average polymorphism, while a value higher than 0.5 indicates a highly polymorphic locus [34]. Based on the PIC values, Lsub01, Lsub10, Lsub11 and Lsub12 were considered as highly performing markers for identification and genetic diversity estimation of garden cress, while Lsub04, Lsub09 and Lsub13 were considered as inefficient markers to scan diversity in the genotypes. High PIC values show that the fragments generated are very informative. Hasan et al. [35] and El-Esawi et al. [36] reported that the PIC demonstrates the informativeness of the SSR loci and their potential to detect differences among the varieties based on their genetic relationships. In the same manner, Shannon's diversity index (I) ranged from 0.26 (for Lsub 04 marker) to 1.41 (for Lsub10 marker) with an average of 0.75 (Table 4), indicating greater genetic diversity among the investigated genotypes. The mean inbreeding coefficient (F_{IT}) across the SSR loci was 0.329. The F_{ST} values ranged from 0.107 (for Lsub01 marker) to 0.566 (for Lsub05 marker), with a mean value of 0.269 (Table 4), indicating very high genetic differentiation among the genotypes.

The high Ho and F_{ST} values in the current study might be due to changes in pollination behavior of the garden cress which is predominantly cross pollinated (under favorable environment) and less self-pollinated (under unfavorable environment). In addition, the presence of both diploid and tetraploid genotypes contributes for high Ho and F_{ST} .

The present study also detected 100% polymorphic loci, which was partially in agreement with the previous studies on garden cress accessions using ISSR markers [8]. Our results are further supported by Kaur et al. [7] in which 82% polymorphism was in agreement with Fayyaz et al. [37] on Brassica population, where higher numbers of fragments for each marker reflected the existence of larger genetic diversity among the investigated genotypes. Markers with high numbers of polymorphic fragments are thus desirable in studying genetic diversity, and discrimination of the genotypes. Based on Nei's test [38],

Table 4
Allele number, effective allele number, Shannon diversity index, heterozygosity and Polymorphism Information Content, Gene flow and F-statistics obtained using SSR markers.

SSR marker	Allele size	AL	AG	BP	Ne	I	PIC	Ho	He	Nei	NM	F _{IT}	F _{IS}	F _{ST}
Lsub01	175-250	4	129	10.8	2.877	1.19	0.652	0.333	0.656	0.652	2.096	0.410	0.473	0.107
Lsub02	175-300	3	127	10.6	1.820	0.862	0.442	0.019	0.453	0.451	0.421	0.909	0.943	0.372
Lsub03	175-250	3	105	8.80	1.463	0.385	0.325	0.018	0.318	0.317	1.180	0.941	0.951	0.175
Lsub04	75-125	2	103	8.60	1.155	0.259	0.134	0.144	0.135	0.134	1.988	−0.192	−0.059	0.112
Lsub05	100-600	2	181	15.1	1.502	0.517	0.334	0.242	0.337	0.334	0.192	0.057	0.590	0.566
Lsub07	75-100	2	137	11.4	1.932	0.675	0.482	0.376	0.485	0.482	2.398	0.211	0.286	0.094
Lsub08	50-125	2	85	7.10	1.926	0.674	0.481	0.000	0.484	0.481	0.290	1.000	1.000	0.463
Lsub09	50-75	2	103	8.60	1.407	0.464	0.289	0.000	0.291	0.289	0.679	1.000	1.000	0.269
Lsub10	175-1000	5	143	11.9	3.628	1.414	0.733	0.647	0.729	0.724	1.762	−0.018	0.108	0.124
Lsub11	200-1000	4	79	6.60	3.261	1.280	0.694	0.689	0.701	0.693	0.281	−0.172	0.381	0.471
Lsub12	50-300	3	130	10.8	2.461	0.976	0.594	0.265	0.597	0.594	1.316	0.499	0.579	0.160
Lsub13	100-125	2	65	5.40	1.202	0.309	0.168	0.185	0.17	0.168	0.559	−0.695	−0.171	0.309
Mean		2.83	115.58	9.63	2.05	0.750	0.444	0.243	0.446	0.443	1.097	0.329	0.507	0.269

AL = Number of alleles per locus; AG = alleles detected per locus across genotypes; BP = Number of bands per primer; Ne = effective number of alleles; I = Shannon diversity index; PIC = polymorphic information content; Ho = observed heterozygosity; He = expected heterozygosity; Nei = Nei's (1973) expected heterozygosity; $NM = 0.25(1-F_{ST})/F_{ST}$ = gene flow among genotypes; F_{IT} = the deficiency or excess of average heterozygotes in a group of populations [−1 (out breeding) to 1 (inbreeding)]; F_{IS} = the deficiency or excess of average heterozygotes in each population [−1 (out breeding) to 1 (inbreeding)]; F_{ST} = the degree of gene differentiation among populations in terms of allele frequencies (0–1).

the highest genetic diversity value of 0.72 was observed from locus Lsub 10, while the lowest value of 0.13 was recorded from locus Lsub 04, with a mean diversity of 0.44 (Table 4). Similar results were reported for genetic diversity analyses of *Brassica napus* and *Brassica oleracea*, using SSR markers by Fayyazet al. [37] and El-Esawi et al. [36], respectively.

3.2. Genetic relationships and cluster analysis

Cluster analysis based on un-weighted neighbor joining method grouped the genotypes into five distinct categories (Fig. 2). All clustered groups consisted of landraces originated from different regions of Ethiopia. Three major clusters (I, III and IV) comprised 39, 38 and 20 genotypes, respectively (Table 5). The remaining two clusters (II and V) contained 10 and 5 genotypes, respectively. Each major cluster was

Table 5
Distribution of Ethiopian garden cress genotypes in five clusters.

Cluster	Genotypes in cluster (Table 1 for codes of accessions)	Number of Genotypes
I	1-18, 21-27, 29-38, 41-43	39
II	20, 28, 39, 44-48, 77, 106	10
III	50, 53, 65, 69, 70, 81, 83, 87-90, 92, 93, 95-105, 107-112	38
IV	19, 49, 51, 52, 54-64, 82, 84, 86, 91, 94	20
V	66-68, 78,85	5

further subdivided into sub-clusters that consisted of members from different region of origin. The first cluster predominantly composed of 12 genotypes from Tigray State and 9 genotypes from Wello Zones in

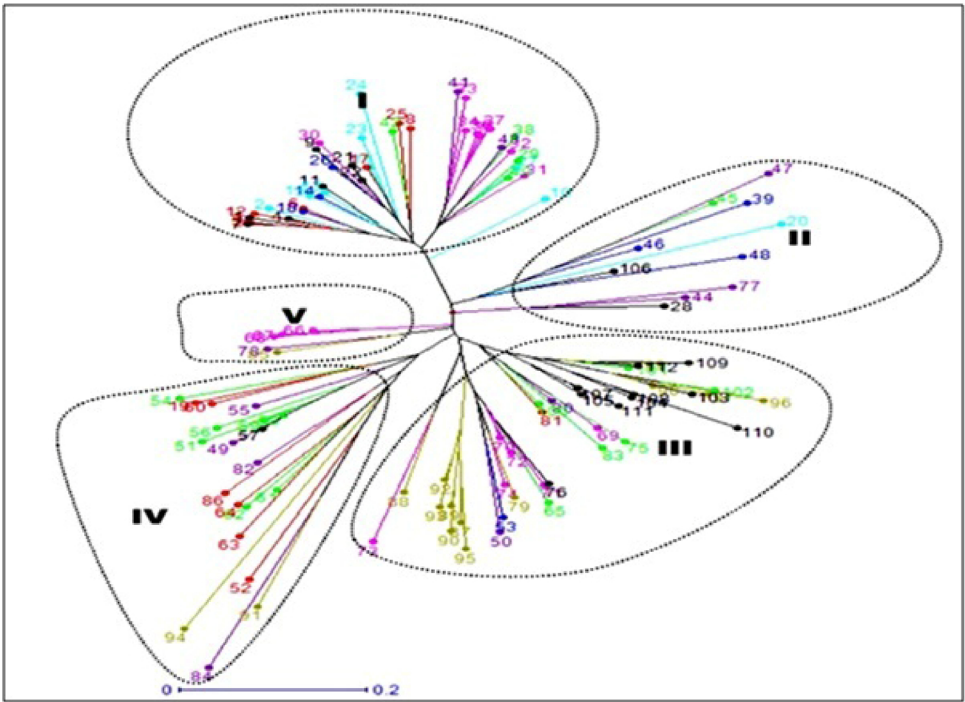


Fig. 2. The grouping of 112 garden cress genotypes into five clusters using unweighted neighbor-joining tree with DARwin 6 software [25]. Colors represent genotypes from the nine populations indicated in Table 3.

Amhara State. About 50% of the genotypes in cluster III were collected from North Omo and Keficho Shekicho in the SNNP State and Tigray State, showing their genetic similarity. However, these two regions are very far from each other. While Keficho Shekicho is situated in the South West of the country, Tigray is over 1200 km away at the farthest north of the country. In Cluster IV, while six accessions were originated from Tigray, seven were collected from Wello (Fig. 2). The cluster analysis indicated genotypes with high level of similarity that might be due to the duplication of genotypes during collection or due to the use of materials with the same background. Genetic relationships were found to be very close within genotypes of each of the three major groups and substantially divergent among the genotypes between the groups which are vital in the exploitation of their variability in future breeding programs.

The weak geographic associations obtained from the present study among the genotypes were similar to the findings of Said and Kassahun [8] on garden cress genotypes using ISSR marker. However, the findings of the present study were in contrary to that of Hajibarat et al. [39] who studied 48 chickpea genotypes using SSR markers which demonstrated the correlation between genotypes diversity and their geographic distribution partly dependent on the type and nature of genotypes used in their study. Generally, the cluster analysis revealed that the grouping of the genotypes did not exhibit defined relationship to the geographical regions of origin, showing random dispersion. This might be due to common ancestry or introduction of the gene pool in the distant past which can reduce genetic differentiation among populations. Similar findings were reported by Said and Kassahun [8] and El-Esawi et al. [36] on the diversity study of garden cress and *Brassica oleracea*, using ISSR and SSR markers, respectively. The cluster analysis, as indicated in unweighted neighbor-joining tree (Fig. 2) were in accordance with the genetic distance (dissimilarity coefficients) ranging from 0.059 to 1.00 with an average of 0.545, signifying high level of molecular diversity among genotypes. The present findings supported previous findings in related species [11,40], where RAPD and SSR markers effectively estimated genetic distances among genotypes of *Brassica* species.

3.3. Principal coordinate analysis (PCoA)

The results of the principal coordinate analysis (PCoA) were compared with the cluster analysis. The first two components explained 22% of the total variation. The distribution and association of genotypes in the three major cluster analysis and PCoA agreed well. In the scattered plot graph (Fig. 3), the genotypes from cluster I and II overlapped and were clearly separated from the genotypes in cluster III, IV and V. The genotypes from cluster I were grouped together, while the genotypes from cluster IV separated away from all other genotypes. PCoA showed that the distribution of genotypes of garden cress was

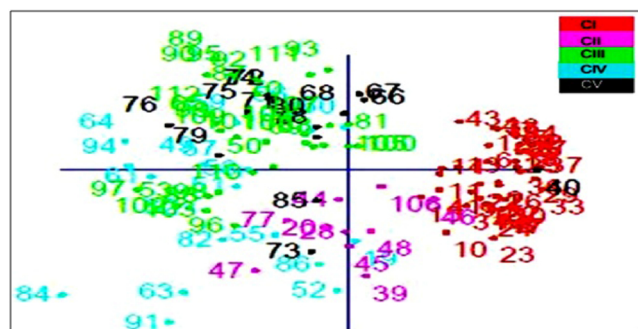


Fig. 3. The scattered ordination plot of the first and second principal coordinate analysis of 112 genotypes clustered into 5 groups with different colors. CI: cluster I; CII: cluster II; CIII: cluster III; CIV: cluster IV; CV: cluster V. Names and number of genotypes under each cluster are shown in Table 5.

Table 6

AMOVA between geographical group (among populations) and the 112 garden cress genotypes (within population).

Source	Degree of freedom	Sum of squares (SS)	Mean Square (MS)	Estimated variance	
				Value	%
Among populations	8	64.948	8.118	0.505	21%
Within population	103	196.115	1.904	1.904	79%
Total	111	261.063		2.409	100%

somehow similar to that observed in the clustered genotypes based on Unweighted neighbor-joining method. Two distinct groups were formed on the axes, however, genotypes such as number 63, 84, and 91 were far from other group members, indicating their unique feature and significant divergence [15]. Most genotypes were grouped in their respective clusters and overlapped with other genotypes with different clusters. They could be distinguished clearly into groups, suggesting a rich genetic variation among genotypes without ignoring appreciable similarity within groups of genotypes (Fig. 3). The results were in agreement with works of Özbek and Gidik [41] on rapeseed.

3.4. The genetic variability within and among populations

The AMOVA for the twelve microsatellite loci and nine populations is presented in Table 6. The deviation from Hardy Weinberg equilibrium (HWE) is an indication of the intensity of various forces acting upon the populations. Majority of the populations except Pop4, Pop6, Pop8, and Pop9 showed deviation from HWE. Likewise, the number of alleles per marker varied from 1.6 (for pop6) to 2.67 (for pop3). The average Nei gene diversity in the populations from 0.18 to 0.503, with an average of 0.340. A total of 98 polymorphic fragments were scored in all populations. The percentage of polymorphic loci indicated that genotypes from Pop2, Pop3, Pop4 and Pop9 had the highest percentage of polymorphic loci (91 to 100%), while the lowest (33 to 50%) were for the genotypes from Pop6, Pop7 and Pop8.

Observed numbers of alleles (N_a) were in the range of 1.6–2.67 across the populations, and effective numbers of alleles (N_e) were higher in Pop4 (1.98) than in other populations. Nei's gene diversity (Nei) was 0.340, and Shannon's diversity index (H) was 0.522 in the tested garden cress genotypes. The mean population percentage polymorphism was 74 varying in the range of 33–100 percent (Table 7). The Shannon diversity index between the populations ($H_t = H_t - H_s / H_t = 0.304$) was smaller than the genetic variation within the population (0.696).

The AMOVA also demonstrated that 79% of the genetic variations resided within populations, while only 21% variations were among the populations (Table 6). These results indicated the presence of relatively wide range of variations among populations, however, there were higher and significant genetic variations within the population than variations among the population. The overall free flow of gene and selection pressures acting on each population as well as the outcrossing nature of the crop have impeded genetic differentiation among populations. The gene flow index ($N_m = 1.097$) indicated that greater genetic exchange across populations altered the effect of genetic drift within populations and prevented the differentiation of populations with $N_m > 1$ [20]. The present study revealed higher genetic variability among genotypes in pop3 and pop4, but lower variability in pop6 and pop7. This information is vital in selecting garden cress genotypes for conservation and improvement. Our results are in agreement with earlier report from garden cress [8]. Similar findings were also reported by El-Esawi et al. [36] in studying genetic diversity in *Brassica oleracea* using SSR markers.

Table 7

The values of genetic diversity parameters of populations estimated based on polymorphisms of 12 SSR loci.

Pop	NG	AP	APP	Na	Ne	I	Ho	He	Nei	NPB	PPB	HWD
Pop1	14	165	13.8	2.30	1.80	0.620	0.201	0.402	0.384	10	83.33	**
Pop2	17	238	19.8	2.50	1.80	0.350	0.287	0.402	0.387	11	91.97	**
Pop3	18	219	18.3	2.67	1.95	0.716	0.205	0.450	0.433	12	100	**
Pop4	8	104	8.70	2.42	1.98	0.712	0.317	0.503	0.454	12	100	NS
Pop5	14	178	14.8	2.33	1.91	0.619	0.227	0.220	0.382	9	75	**
Pop6	10	114	9.50	1.60	1.43	0.310	0.133	0.210	0.197	4	33.3	NS
Pop7	15	136	11.3	1.67	1.41	0.300	0.170	0.192	0.18	5	41	**
Pop8	7	102	8.50	2.00	1.74	0.455	0.236	0.293	0.271	6	50	NS
Pop9	9	131	10.9	2.42	1.75	0.617	0.343	0.401	0.372	11	91.6	NS
Total	112	1387	115.57	16.42	13.56	3.705	2.12	2.552	2.384	98	87.5	
Mean		154.1	12.843	2.212	1.752	0.522	0.235	0.341	0.340	8.89	74.03	

Pop = population; NG = Number of genotypes; AP = amplified alleles detected per population; APP = Number of amplified alleles per primer; Na = allelic richness; Ne = effective number of alleles; I = Shannon diversity index; Ho = observed heterozygosity; He = expected heterozygosity; Nei = gene diversity; NPB = Number of polymorphic band per primer; PPB = percentage of polymorphic band; HWD = Hardy-Weinberg disequilibrium. NS = not significant.

Table 8

Nei's unbiased measures of genetic distance (below diagonal) among populations (pop) using 12 SSR primers.

	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Pop7	Pop8
Pop1								
Pop2	0.122							
Pop3	0.067	0.077						
Pop4	0.212	0.123	0.130					
Pop5	0.191	0.106	0.079	0.109				
Pop6	0.246	0.259	0.188	0.284	0.243			
Pop7	0.372	0.330	0.302	0.333	0.311	0.291		
Pop8	0.270	0.100	0.129	0.084	0.042	0.258	0.396	
Pop9	0.306	0.158	0.190	0.038	0.090	0.281	0.327	0.044

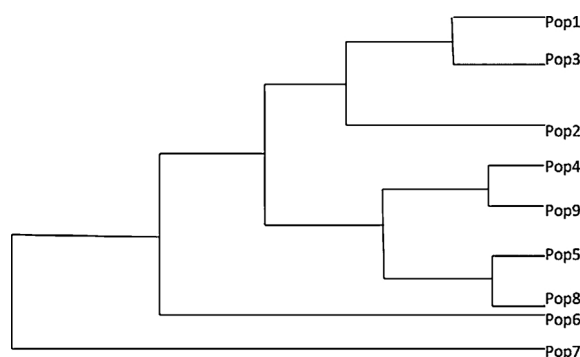


Fig. 4. Dendrogram showing the genetic relationships between populations using UPGMA analysis. Pop refers to population. The numbers of genotypes and areas of collections for each population are indicated in Table 3.

3.5. Genetic distance

The genetic similarity coefficients and UPGMA (unweighted pair group method with arithmetic mean) dendrogram have revealed varying degrees of genetic relationships based on geographic groups of origin. The unbiased genetic distance among the sources of origin estimated using Dice's coefficient [26] ranged from 0.038 (between Pop4 and Pop9) to 0.396 (between Pop7 and Pop8), showing wide range of dissimilarity between populations (Table 8). Generally, the cluster analysis indicated that useful genotypes could be selected for breeding program from the three major groups (namely, Pop7, Pop6 and the remaining seven populations: Pop1, Pop3, Pop2, Pop4, Pop5, Pop8, and Pop9) (Fig. 4). Comparatively lower genetic distance between majority of populations were observed. The constructed matrix revealed that least genetic distance (0.038) was noted between Pop9 and Pop8. Genotypes from Gondar (Pop8) and from Jimma, Keficho Shekicho and North Omo (pop7) were the most divergent (0.396)

(Fig. 4), hence could be used as parental lines in the hybridization programs.

4. Conclusions

There is very limited genomic information on the Ethiopian garden cress. The crop is cultivated by subsistence farmers in Ethiopia where only landraces are cultivated in the country since no improved variety has been so far been released. That is the reason why the crop is considered as an orphan or neglected crop. This study is the first step towards the characterization of garden cress germplasm using micro-satellite markers. A successful attempt has been made to examine genetic diversity of Ethiopian garden cress using 12 SSR markers. The study has detected high level of polymorphism, demonstrating a high level of genetic variation among the analyzed 112 genotypes. All selected markers amplified a total of 1387 alleles with an average of 116 alleles per locus, showing all loci polymorphisms. The cluster analysis grouped the genotypes into five clusters independent of their geographic origin. A wider molecular diversity was noted among the genotypes rather than among the populations. The results of this study will provide useful information for future breeding programs and for the evaluation and conservation of these genetic resources. Nevertheless, for in-depth characterization of the garden cress germplasm and to develop useful molecular markers for key agronomic traits, large number of highly polymorphic and species specific SSR markers need to be developed.

Conflict of interest statement

The authors have no conflict of interest.

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